Assessment of Superworm (*Zophobas morio* L.) (Coleoptera:Tenebrionidae) on Different Diets Fermented with Probiotics

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Abstract: Superworm (*Zophobas morio*) is a valuable protein source, offering high nutritional content, making it an essential feed for poultry, while also representing a sustainable alternative protein option for various industries. Evaluation of diets supplemented with different levels of probiotics on developmental stages and nutritional profile of super worm (Z*ophobas morio* L) was carried out under lab condition at 25.5 ±3°C and 60±5% R.H and photoperiod of 14:10 (light/dark). Superworms were feed with wheat bran containing different probiotics, each having three levels. Four probiotics, two bacterial probiotics (*B. clausii* and *L. rhamnosus*) and two fungal probiotics (*C. indica* and *S. cerevisiae*) were used. Experiments comprised of 13 diets, arranged in a completely randomize design (CRD) with three replications. Each diet was made by adding given quantity of probiotics per kg of wheat bran. *B. clausii* @2ml ,4ml and 6ml, L. *rhamnosus* @0.2 g, 0.4g and 0.6g, *C.indica* @25g , 50g and 75g, *S. cerevisiae* @50g, 100g and150g and standard diet (wheat bran only). Results revealed that developmental stages of *Z. morio* were significantly affected by tested diets. In general, wheat bran (standard diet) was found more effective had maximum larval, pupal and adult weight and size followed by fungal probitics (*C. indica* and S. *cerevisiae)* and bacterial probtics (*B. clausii* and *L. rhamnosus*). Results further revealed that *C. indica* and S. *cerevisiae-based* diet promoted quick larval growth (81–89 days) and (78–81 days) respectively compared to *B. clausii* (94-106 days) and *L. rhamnosus* (90-91 days). Results further showed that no larval and pupal mortality was recorded in all tested diets including standard diets. Nutritonal profile of *Z.morio* showed that all probiotic based diet enhanced protein content of *Z. morio* larvae, particularly fungal based probiotic, where protein content was (63-67%) compared to standard diet (59.33 %). Whereas fat and ash content of *Z. morio* lavae reared on protiobic based diets were lower than standard diet. It is concluded that addition of higher dose of probiotic, particularly *C. indica* and S. *cerevisiae* in wheat bran could lead to improvement in mass rearing and protein content of *Z.morio* larvae.

Key words: Super worm, Diets, Bacteria and Fungus

INTRODUCTION

The world population is expected to be nine billion by 2050 (Kroncke *et al*. 2019). This significant increase in world human population increases demand for food resources especially

meat animal protein (Markkar *et al.,* 2014). To fulfil food demands for poultry and livestock with in available limited resources is a big challenge which needs to be addressed. Currently poultry and fish industry use soybean as protein source in poultry and fish Because of their strong digestibility and protein content, they make excellent feed (including a different amino acid profile) (Makkar *et al.,* 2014). In the recent decade, there has been a significant increase in the price of poultry feed ingredients, particularly soya bean. This high cost of soybean and disruption in the feed industry's ability to meet predicted demand for meat and other products is expected to be threatened by a lack of feed component availability. This has prompted nutritionists to look for alternate protein sources that are both cheaper and nutritionally equivalent to other regularly used protein meals in poultry diets (Okah and Onwujiariri, 2012).

In recent years, insects have received much attention in the list of potential protein sources to increase autonomy in poultry feed. In poultry feed, it is utilized in both dry and fresh forms (Khan, 2018). Live (fresh) insects, according to some poultry nutritionists, are a natural way to feed chickens and have attracted increased attention as an effective and sustainable alternative protein. Furthermore, insects also have high fats content, lauric acids and antimicrobial peptides that promote chicken health (Gasco *et al*.2018).

The superworm larvae (*Zophobas morio* L.), also known as darkling beetles (Tenebrionidae), are larger than meal worms (5–6 cm at the end of the larvae stage). It can easily be raised on a substrate that contains a variety of organic wastes, including garden and vegetable wastes (Harsanyi et al., 2020*).* Super worm has recently been identified as a significant and potentially valuable source of protein and lipid in poultry feed (Benzertiha *et al*., 2020; Kierończyk *et al*., 2018). It contains a high amount of crude protein (44–47 % dry matter) and lipid (40–41 % dry matter), as well as important amino acids, fatty acids, and antimicrobial peptides (Benzertiha *et al*., 2020; Nederlof *et al*., 2017; Soon *et al*., 2018). In China and some european countries it is commercially produced and used as protein source for poultry and fish meal (Van Broekhoven, 2015). It has the potential to replace soybean and fishmeal as a protein source in poultry diets (Calislar, 2017). However, fresh insects with a high-water content could be susceptible to degradation and microbial activity (Kroncke *et al.,* 2019). Thus, super worm in dry form found to be suitable for feed production. According to research, insect-based meals are comparable to fish and soybean meal (Huis *et al*., 2013).

Super worm is easy to breed, inexpensive and produce less harmful effects on the environment (Wang *et al*. 2010). Van Huis (2013) studied the environmental impact of conventional livestock compared to insect production. According to reports, cattle require 7.7 kg of feed, sheep 6.3 kg, and pig 3.6 kg; poultry require only 2.2 kg, and insects require only 1.7 kg of cricket meal to create 1 kg of protein. Insect production appears to be the most environmentally friendly species in terms of protein production. According to (Biasato *et al*., 2016). The inclusion of super worm in poultry diets did not influence the physiological changes, thus suggesting that super worm can safely be used in chicken diets.

In the last decade, the usage of probiotics in poultry feed has increased due to European Union has placed restrictions on the use of antibiotics for prophylactic. Alternative ways for

preventing infections during insect mass raising are being developed. Inclusion of Probiotics bacteria to the diet is one such solution. (Cogliani *et al*., 2011). Probiotics reduce increasing animal performance and preventing disease The bulk of probiotics currently in use, however, the lactic acid bacteria, primarily *Enterococcus sp*. and *Lactobacillus sp* Because the maggots are processed without removing the gut, the remaining microbiota is carried over to the ultimate consumer, adding Probiotics bacteria to super worm diets is also beneficial. Up to 10% insect biomass is made up of bacteria (Douglas, 2015). The Probiotics bacteria are known to produce health-promoting metabolites such as Vitamin b, they enhance the nutritional value of meal worms. (LeBlanc *et al*. 2013). On the nutritive value, there is conflicting information of super worm meal worm diet fermented with probiotics. The present study is an attempt to see the response of super worms to diet fermented with Probiotics bacteria in terms of growth, performance, and nutritional profile. If the demand for insects rises dramatically in the future, new production techniques (for mass-rearing) will be required. To be successful in commercial insect farming, new diets and techniques must be developed to full fill the requirement of protein for poultry feed.

MATERIALS AND METHODS

Experiment on evaluation of probiotics supplemented diets on developmental stages and nutritional profile of super worm (*Z. morio*) was carried out at the Insect Rearing Laboratory, Department of Entomology, The University of Agriculture Peshawar during 2021.

Establishment of a super worm stock culture

Wheat bran was purchased from a local market and were sterilized in the autoclave (Model No. HL-340) at 12l°C for 30 minutes. After sterilization, the wheat bran was sieved through sieve No. 14 to make it desirable for larval consumption under environmental incubation chamber (BJPX-A15000) at 25.5 ±3°C and 60 ±5% relative humidity and photoperiod of 14:10 (light/dark). Early instar larvae of super worm were placed in rearing tray (17cm long 11cm wide 7cm deep) and provided with wheat bran. Potatoes were cut in pieces and were placed in rearing tray over the wheat bran, as moisture source. Growing super worm was provided with fresh wheat bran and potato on weekly basis. This process wascontinued till end of the study

Diets used in the experiments.

Four probiotics, two bacteria ospor (*Bacillus clausii*), imutec (*Lactobacillus rhamnosus*) and two fungi (*Calocybe indica*), yeast *(Saccharomyces cerevisiae)* were purchased from local market and added with wheat bran (standard diet) in different levels. Detail of the diets *i.e* D1 (*B. clausii2ml),* D2 (*B. clausii 4ml),* D3 (*B. clausii 6ml),* D4 *(L. rhamnosus 0.2*), D5 *(L. rhamnosus 0.4g),* D6 *(L. rhamnosus 0.6g),* D7 *(C.indica 25g),* D8 (*C.indica 50g),* D9 *(C.indica 75g),* D10 *(S. cerevisiae 50g),* D11 *(S. cerevisiae 100g),* D12 *(S. cerevisiae 150g),* D13, (Wheatbran (Standard diet) 500g). Diets combination= probiotics added per kg of wheat bran

The 30 individuals super worm larvae were collected from the stock culture of super worm and placed in a Petri-dish (35 × 10 mm). Larval development was monitored daily tilled adult emergence.

DIET PREPARATION

The super worm diet wasmade from four different sources and probiotics, Ospor and imutec *(B. clausii and L. rhamnosus),* fungi (*C. indica*), yeast *(S. cerevisiae).* wheat bran and probiotics were purchased

from local market. Wheat bran used in the experiment was sterilized in an autoclave (Model No. HL-340) at 12l°C for 30 minutes to kill all microorganisms. Following sterilization, the diet was grounded and sieve through sieve no. 14 to make it suitable for larval consumption. Required number of probiotics were added in wheat bran before offer to super worm as mentioned in table 1-3. Healthy super worm early instar (30) larvae were collected from stock culture and placed in plastic box (17cm long 11cm wide 7cm deep) and provided with 50 grams of each diet separately. Potato cubes was placed on top of each box to serve as a moisture source and boxes were kept in Environmental Chamber (Model No. 78532) at 25.5°C, with 60~65% relative humidity and photo-period of 14:10 (Light: Dark) for further study.

Parameters

Experiment was arranged in a completely randomize design (CRD), having 13 treatments and each with three replications. Data were recorded at the following parameters.

Larval life span (days)

Larval duration was recorded on randomly selected 10 larvae in each diet at 12 hours interval then average larval duration were calculated.

Larval body weight (mg)

Larval weight was recorded by randomly selecting 10 larvae from each diet separately and were weighed by using electric balance than average larval weight was calculated.

Larva size (mm)

Larval size was recorded by randomly selected 10 larvae from each diet. Image of the selected larvae was captured by using a Nikon SMZ 745T trinocular stereomicroscope mounted with a Nikon FSi2 and then measured using the ImageJ software (version 1.8.0) to reared the average larval size.

Percent larval mortality

Larval mortality in each diet was calculated by counting the number of dead and live larvae then % percent larval mortality was calculated by using the following formula.

$$Larval\ mortality\ \% = \frac{dead\ larva}{Total\ no\ of\ larvae} \times 100$$

Pupal duration (days)

Pupal duration was recorded on randomly selected 10 pupae in each diet at 12 hours interval then average pupal duration were calculated.

Pupal weight (mg)

Pupal weight was recorded by randomly selecting 10 pupae from each diet and were weighed by using electric balance separately than average pupal weight was calculated.

Pupal size (mm)

Pupal size was recorded by randomly selected 10 pupae. Image of the selected pupae was captured by using a Nikon SMZ 745T trinocular stereomicroscope mounted with a Nikon FSi2 and then measured using the ImageJ software (version 1.8.0) to reared the average pupal size.

Percent Pupal mortality

Pupal mortality in each diet was calculated by counting the number of dead and live pupae then percent pupal mortality was calculated by using the formula:

$$\%\ Pupal\ mortality = \frac{total\ dead\ pupa}{Total\ no\ of\ pupa} \times 100$$

Adult longevity (days)

Adult longevity was recorded for each diet from time of adult emergence from egg till death.

Adult weight (mg)

Adult weight was recorded by randomly selecting 10 mature adults from each diet and was weighed by using electric balance separately than average adult weight was calculated.

Adult size (mm)

Adult size was recorded by randomly selected 10 full grown adults. Image of the selected adult was captured by using a Nikon SMZ 745T trinocular stereomicroscope mounted with a Nikon FSi2 and then measured using the ImageJ software (version 1.8.0) to reared the average adult size.

Adult sex ratio

Male and female adults of *Z. morio* were separated from the entire adult population of each diet separately then % sex ratio was calculate by using formula.

$$\%\ sex\ ratio = \frac{total\ no\ of\ male\ adult\ on\ diet}{Total\ no\ of\ adults} \times 100$$

Nutritional profile

Biochemical analysis of super worm was carried out Biochemistry chemistry Lab at Veterinary Research Institute (VRI), Peshawar. Healthy late instar larvae of Super worm were collected from their respective diets. The collected larvae were dried through dryer. After drying the larvae of super worm were grinded through electric grinder to make a fine powder and were kept in tagged plastic bottle for chemical analysis.

Crude protein (Kjeldahl method), crude fat (Soxhlet method) and ash contents (muffle furnace) will be determined by standard procedure as mentioned in AOAC 1990, AOAC, 2003, and AOAC, 2013 respectively.

Protein Content

The protein analysis was done on the basis of live weight. An electric balance is use for to measure the live weight of the larvae in each diet. After that, the worms were freezed and dried in the freezer for 48 hours. A laboratory grinder is use to pulverize each diet larvae. The pulverized material was place in digesting tubes. In a digestion block heater, the samples were digested at 420°C for 30 minutes. After removing the digesting tubes and allowing them to cool for 10 minutes, 30 mL of distilled water were added to each of them. The tubes were place in the Auto Analyzer, together with the digests. Distillation, titration, and protein calculation was done automatically. The protein percentage computed from the following equation:

Protein Content = Displayed results ÷ sample weight of live worms (mg)

Fat Content

The fat content is determining based on live weight. In Each group the live weight was recorded on electric balance. The worms werefreeze in the refrigerator for 48hand dried. A laboratory grinder was used to grind each group. The fat content was evaluated using an ether

extraction technique specific in the Association of Official Analytical Chemists' Official Method (2003). A permeable jar filled with grounded super worm is used to transfer the hot ether through. The fat is separated from the dried particles and collected in a flask at the apparatus's bottom. Remove the container and dried for 24 hours at 105°C in an oven (Isotemp oven, model 655F, VRI, Peshawar). The weight loss is proportional to the initial sample's fat content. The fat percentage was calculated using the following formula:

Fat Content = extracted fat weight (g) ÷ sample weight of live worms (g) × 100

Ash Content

The dried worms were placed in a muffle furnace (Isotemp muffle furnace, Model No. 186A, Veterinary Research Institute (VRI) Peshawar) for 30 min at 550°C. They were removed, then left to cool in a desiccator and weigh using an electric balance (SMZ). The ash content calculation as follows:

Ash Content =Ash weight after burning of worms (mg) ÷ live worms' weight (mg) × 100

Statistical Analysis

The designs of the experiments were completely randomized designs (CRD) and data was subjected to Analysis of Variance (ANOVA) by using statistical software (Statistix 8.1). Means wascompared with using Least Significant Difference (LSD) Test at 5% probability level.

RESULTS

4.1 Effect of different diets fermented with probiotics on larval parameters of *Z. morio*

Results regarding larval parameters of *Z. morio* is presented in Table 1. It was found that larval parameters other than larval mortality was significantly affected when offered different diets fermented with probiotics.

Significant variation in *Z. mori* larval weight was observed (df = 12, F value = 56.0, P value = 0.000), when offered different diets. However, larval weight was maximum (914.7 mg) when fed on diet contain *S. cerevisiae*150g followed by the diet having *S. cerevisiae 100* g (913.5mg) and standard diet (913.2mg). whereas minimum larval weight was recorded in case of *B.clausii* 2ml (899.5 mg). Results further revealed that fungus-based diets performed better having more healthy larvae than bacterial based diets. Larval weight of *Z. mori* increased when concentration of the *C. indica* and *S. cerevisiae* in the diets increased. Larval weight recorded in*C.indica* 25 gm, 50 gm and 75 gm were907.7 mg, 909.8 mg and 910.3 mg respectively. Where as in case of *S. cerevisiae*based diet, larval weight was 9.118 mg, 913.5 mg and 914.7 mg recorded in diet containing *S. cerevisiae*50g, 100g and 150 gm respectively.Results also showed that among bacterial diets,*B. clausii*based diet yielded lower larval weight ranging from (899.05 mg – 901.5 mg) than *L. rhamnosus*where larval weight was significantly higher 906.5 mg, 907.4 mg and 908.2 mg on diets having 0.2g, 0.4 gm and 0.6 gm *L. rhamnosus* respectively.

Results of larval size shows that larval size was significantly affected by different diets (df = 12, Fvalue = 39.5, P value = 0.000) However, larval size was maximum (94.5 mm) on diet contain *S. cerevisiae* 150g followed by the diet having *S. cerevisiae 100* g (90.7 mm) and standard diet (90.9 mm) whereas minimum larval size was recorded in case of *B. clausii* 2ml (75.8 mm). Overall*B. clausii and L. rhamnosus* produced smaller larvae than *S. cerevisiae*and*C.indica*. Results further show that concentration of the*B. clausii*and *L. rhamnosusin* in the diet increased the larval

size increased and vice versa.

As presented in Table 4.1 larval duration was maximum (106 days) in *B. clausii* 2ml (106 days) followed *B.clausii* 4m (91.5 days), *B.clausii* 6ml (94 days), *L.rhamnosus*0.2g (91.5 days), *L. rhamnosus* 0.4g(94 days), *L. rhamnosus* 0.6g (90.5 days) while minimum larval duration was recorded

As far as larval mortality is concern, no larval mortality was recorded in all tested diets. It shows that all tested diets were 100 safe and no adverse effect of larva stage of *Z. morio.*

Table 1. Effect of different diets fermented with probiotics on larval parameters of *Z. morio* under lab condition

Diets	Larval parameters				
	Diets Combination	**Larval weight (mg)**	**Larval size (mm)**	**Larval duration (days)**	**Larval mortality (%)**
D1	*B. clausii* 2 ml	899.5 h	75.8 h	106 a	0.00
D2	*B. clausii* 4 ml	901.5 g	77.0 h	91.5 b	0.00
D3	*B. clausii* 6 ml	901.1 gh	80.7 g	94 b	0.00
D4	*L. rhamnosus*0.2 g	906.5 f	80.9 g	91.5 b	0.00
D5	*L. rhamnosus*0.4g	907.4 f	82.5 fg	94 b	0.00
D6	*L. rhamnosus*0.6g	908.2 ef	84.0 ef	90.5 bc	0.00
D7	*C.indica* 25g	907.7 f	86.7 cd	81 d	0.00
D8	*C.indica* 50g	909.8 de	86.1 de	87 c	0.00
D9	*C.indica* 75g	910.3 cd	86.9 cd	89.5 bc	0.00
D10	*S. cerevisiae* 50g	911.8 bc	88.9 bc	81.5 d	0.00
D11	*S. cerevisiae* 100g	913.5 ab	90.7 b	80.5 d	0.00
D12	*S. cerevisiae* 150g	914.7a	94.5 a	78.5 d	0.00
D13	Wheat bran (Standard diet)	913.2 ab	90.9 b	90.5 bc	0.00
LSD (0.05)		**1.9072**	**2.5839**	**4.1497**	**0.00**

*Diets combination= mg / ml of probiotics added per kg of wheat bran

4.2 Effect of different diets fermented with probiotics on pupal parameters of *Z. morio*

Results regarding the effect of different diets on pupal parameters of *Z. morio* are presented in Table 2. Results showed that pupal weight, pupal size, pupal duration were significantly affected by different diets except pupal mortality, which was zero in all diets.

Standard diet was found the best having maximum pupal weight (879.37 mg) followed by diet contain *S. cerevisiae* 50 mg and 150 mg (874.50 mg and 875 .33 mg) respectively while *B. clause* 2 ml was found the least effective diet having the lowest pupal weight (855.37 mg). Pupae in case of bacterial based diets, (*B. clausii* and *L. rhamnosus*) was comparatively lighter, ranging from (855.37 mg – 871.56 mg) than *C. clausii* and *L. rhamnosus* with pupal weight ranging from (868.49 mg - 879.37 mg).

Similarly bigger pupae were recorded in case in standard diet (84.12 mg) followed by diet having *S. cerevisiae* 100 g (80.30 mm) while smaller pupae were observed in *B. clausii* 2ml (60.51 mm). Diet having *B. clausii* and *L. rhamnosus* produced smaller pupae than the rest of diets including standard diet.

As far as pupal duration is concern, *Z. morio* spent mimimum time (12.16 days) as pupae on standard diet followed by *C. indica* and *S. cerevisiae-based* diets with pupal duration duration of (13.50-14.14 days). While longest pupal duration was recorded in *B.clausii* 2ml (20.19 days). Among four probiotics, *B. clausii and L. rhanmosus* based diet prolonged pupal duration of *Z.morio*as compared to *C. indica* and *S. cerevisae.* Result regarding effect of different diets on percent pupal morality shows that pupal mortality was not significantly affected by tested diets. However, no pupal mortality (0%) was recorded in all tested diet.

4.3 Effect of different diets fermented with probiotics on adult parameters of *Z. morio*

Results regarding the effect of different diets fermented with probiotics on different adult weight, adult size and adult longevity of *Z. morio* are presented in Table 3. Results revealed that the tested diets had significant effect on adult parameters.

Significant variation in adult weight was recorded. However, maximum adult weight was recorded in standard diet (102.8 mg) followed by *S. cerevisiae* at 150g (100.8 mg), *C. indica* 75g(100.3mg), *S. cerevisiae* 50 g (99.5mg), *S. cerevisiae* 100 g (98.5 mg) and *C. indica* 25g and 50 g(98.3mg) while the minimum adult weight was recorded in *B. clausii* based diet particularly *B. clausii* 2ml (83.9 mg). Adult weight recorded in *L. rhamnosus* based diets were ranging from (90.3 – 96.7 mg) these were significantly higher than *B. clausii* based diets but significantly lower than *C. indica* and *S.cerevisiae* as well as standard diet. Similar trend was observed in case of adult size. Adult size was maximum (107.2 mm) in wheat bran (standard diet) followed by *C. indica* and *S. cerevisiae-based* diet (96.3 mm - 98.3 mm) while minimum adult size was recorded in *B. clausii* (80.2 mm). In general *C. indica* and *S. cerevisiae-based* diet produced bigger beetle than *B. clausii* and *L. rhamnosus* based diet.

Adult longevity was significantly affected by larval diets. Adult longevity was maximum in standard diet (101.8 days) followed by *S. cerevisiae* at 50g, 100g and 150g with 95.8 days, 96.1

and 98.1 days respectively. Where minimum adult longevity was recorded in *B. clausii* based diets ranging from 86.1 – 88.6 days at 2ml, 4ml and 6 ml respectively.

4.3 Effect of different diets fermented with probiotics on sex ratio (%) of *Z. morio*

Results presented in Table 4 shows that sex ratio of *Z. morio* recorded in different diets. Results revealed that irrespective of diets, adult male was dominated over female. However, highest percentage of males was recorded in standard diet and *B. clausii* 6ml based diet 56% while the lowest percentage of males were recorded in of diet having *B. indica* 25 gm. whereas female percentage was equal to and below 50% in all diets. The lowest female percentage is recorded in standard diet that is 44% while highest female percentage was recorded in case of diet having B. *indica* 25 gm.

Table 2. Effect of different diets fermented with probiotics on pupal parameters of *Z. morio* under lab condition.

Diets	Pupal parameters				
	Diets Combination	Pupal weight (mg)	Pupal size (mm)	Pupal duration (days)	Pupal mortality (%)
D1	*B. clausii* 2 ml	855.37 h	60.51 i	12.16 f	0.00
D2	*B. clausii* 4 ml	861.39 g	62.53 hi	12.96 ef	0.00
D3	*B. clausii* 6 ml	861.41 g	65.69 g	12.83 ef	0.00
D4	*L. rhamnosus*0.2 g	868.12 e	65.32 gh	13.54 def	0.00
D5	*L. rhamnosus*0.4g	865.37 f	69.14 f	14.14 c-f	0.00
D6	*L. rhamnosus*0.6g	869.52 de	70.30 ef	14.50 b-e	0.00
D7	*C.indica* 25g	870.22 cd	72.52 de	14.50 b-e	0.00
D8	*C.indica* 50g	868.49 de	73.15 de	13.50 d-f	0.00
D9	*C.indica* 75g	871.56 c	74.87 cd	16.50 b	0.00
D10	*S. cerevisiae* 50g	875.33 b	74.23 cd	15.16 b-d	0.00
D11	*S. cerevisiae* 100g	871.51 c	80.30 b	16.50 b	0.00
D12	*S. cerevisiae* 150g	874.50 b	76.61 c	15.68 bc	0.00
D13	Wheat bran (Standard diet)	879.37 a	84.12 a	20.19 a	0.00
LSD (0.05)		**1.765**	**2.889**	**2.110**	**0.00**

*Diets combination= mg / ml of probiotics added per kg of wheat bran

Table 3. Effect of different diets fermented with probiotics on adult parameters of *Z. morio* under lab condition.

Diets	Adult parameters			
	Diets combinations	Adult weight (mg)	Adult size (mm)	Adult longevity (days)
D1	*B. clausii* 2 ml	83.9 e	80.2 d	101.8 a
D2	*B. clausii* 4 ml	87.2 de	80.2 d	98.1 b
D3	*B. clausii* 6 ml	89.0 de	86.5 c	95.8 bc
D4	*L. rhamnosus*0.2 g	90.3 cd	87.2 c	94.5 c
D5	*L. rhamnosus*0.4g	96.7 b	89.0 c	93.6 cd
D6	*L. rhamnosus*0.6g	96.36 bc	97.6 b	96.1bc
D7	*C.indica* 25g	98.3 ab	89.5 c	94.1 c
D8	*C.indica* 50g	98.3 ab	96.3 b	90.5 de
D9	*C.indica* 75g	100.3 ab	96.6 b	89.1 ef
D10	*S. cerevisiae* 50g	99.5 ab	97.6 b	88.1 ef
D11	*S. cerevisiae* 100g	98.5 ab	98.3 b	86.1 f
D12	*S. cerevisiae* 150g	100.8 ab	98.3 b	88.6 ef
D13	Wheat bran (Standard diet)	102.8a	107.2 a	86.5 f
LSD (0.05)		**6.200**	**4.770**	**3.431**

*Diets combination= mg / ml of probiotics added per kg of wheat bran

Table 4 . Effect of different diets fermented with probiotics on sex ratio (%) of *Z. morio* under lab condition.

Diets	Sex ratio (%)		
	Diets combination	Male ♂	Female ♀
D1	*B. clausii* 2 ml	53	47
D2	*B. clausii* 4 ml	52	48
D3	*B. clausii* 6 ml	56	44
D4	*L. rhamnosus* 0.2 g	51	49
D5	*L. rhamnosus* 0.4g	55	45
D6	*L. rhamnosus* 0.6g	54	46
D7	*C.indica* 25g	50	50
D8	*C.indica* 50g	51	49
D9	*C.indica* 75g	54	46
D10	*S. cerevisiae* 50g	51	49
D11	*S. cerevisiae* 100g	55	45
D12	*S. cerevisiae* 150g	53	47
D13	Wheat bran (Standard diet)	56	44

*Diets combination= mg / ml of probiotics added per kg of wheat bran

Table 5. Effect of different diets fermented with probiotics on proximate composition of *Z. morio* under lab condition

Diets	Proximate composition of super worm			
	Diets combination	Protein%	Crude Fat%	Ash%
D1	*B. clausii* 2 ml	62.60 ef	14.0 b-d	7.10d
D2	*B. clausii* 4 ml	62.00 f	14.5 bc	7.07 d
D3	*B. clausii* 6 ml	62.72 d-f	14.1 b-d	7.13 d
D4	*L. rhamnosus*0.2 g	62.41 ef	14.8 b	7.28bc
D5	*L. rhamnosus*0.4g	62.35 ef	14.4 b-d	7.34 b
D6	*L. rhamnosus*0.6g	63.29 c-e	14.8 b	7.35b
D7	*C.indica* 25g	63.81 b-d	13.5 d	7.18 cd
D8	*C.indica* 50g	64.70 b	13.7 cd	7.15d
D9	*C.indica* 75g	64.06 bc	13.7 cd	7.06d
D10	*S. cerevisiae* 50g	64.62 b	13.8 cd	7.09 d
D11	*S. cerevisiae* 100g	68.56 a	8.8 e	6.5 e
D12	*S. cerevisiae* 150g	67.65 a	8.1 e	6.4 f
D13	Wheat bran (Standard diet)	59.33g	16.1 a	7.71 a
LSD (0.05)		**1.56**	**0.98**	**0.123**

*Diets combination= mg / ml of probiotics added per kg of wheat bran

4.5. Effect of different diets fermented with probiotics on proximate composition of *Z. morio*

Nutritional profile (protein content, crude fat and ash content) of dried *Z. morio* larvae reared on different diets is shown in Table 5. Nutritional profile of *Z. morio* varied significantly on different diets.

Results regarding protein content of *Z. morio,* fed on different diets shows that in general, protein content was higher in *S. cerevisiae* based diet (64.62% - 67.65%) followed by *C. indica* based diet, *L. rhamnosus* based diet, *B. clausii* based diets with protein content of (63.81 % – 64.06%), (62.41% - 63.29%), (62% - 62.72%) respectively. while lower protein content was recorded in control (59.33%).

As far as the fat content is concern, significant variation in fat content in *Z. morio* was recorded when fed on different feeding regimes. Highest fat content was recorded in standard diet (wheat bran) 16.1 % while lowest 8.1% was recorded in S. cerevisiae 150gm. In general, *Z. mori* larvae feed on bacterial based diets *B. calusii* and *L. rhamnosus* had higher fat content than *C. indica* and *S. cerevisiae*-based diets.

Similarly, ash content was significantly affected by different diets offered to *Z. mori*. Ash content was higher in standard diet 7.71% and lower in *S. cerevisiae* 150g (6.4%).

DISCUSSION

Insects have recently been identified as an importrant protrin source for animal and poultry birds feed (Premalatha et al., 2011) Many insects have been report as rich source of protein such as Crickets, grasshopper, caterpillar, several beetles and houseflies by weight compared to beef, pork, chicken, and lamb (Srivastava *et al*., 2009).

The darkening beetle commonly known as super worm (*Z. morio*) is considered an important source of protein feed not only for animals but also for humans and they are used for different life support systems (van Huis. 2013, Finke. 2002; Li *et al.*,2012 and 2015).

The European Union has ban on the use of antibiotic in farm animals and poultry birds in 2006, eliminating the use of antibiotics has considerable consequences on animal performance and increased incidence of animal diseases (Bajagai *et al.*, 2016). Diet composition play an important role in insect development and the presence of probiotics in insect diet can protect different insects from pathogens. The role that probiotic bacteria and micronutrients play in enhancing insects' fitness and immune responses in presence of pathogens infection needs more investigations. EU restrictions on the prophylactic use of antibiotics have encouraged the development of alternative strategies to prevent infections during the mass rearing of insects, and the addition of probiotic bacteria to the diet is one such approach (Cogliani *et al* .2011)

Insect diet has profound effect on performance and development of insect (Yuval *et al*.2002). Some earlier researchers has reported that wheat bran as standard diet for mass rearing of darkening beetles meal worm and superworm. In the present study different probiotics added in wheat bran were evaluated for rearing *Z.morio*. Results revealed that *Z. morio* was successfully reared on diets fermented with probiotics, without any larval and pupal mortality. The findings of the present study agree with those of Zhong *et al*. (2017), who clearly demonstrated the exploitability of a probiotic feed additive for feeding of yellow mealworms. Vesna *et al*. (2021) also observed no mortality *T. molitor* larvae treated with probiotic strains of *B. clausii* throughout

the 7-day rearing period, thus suggesting that the occurrence of *B. clausii* in the rearing substrate did not affect the viability of yellow mealworms. Wan et *al*. (2016) reported that probiotic bacteria increase the fitness of the host. The addition of probiotic bacteria to mealworm diets would therefore generate an insect-based feed that contained both prebiotics and probiotics for livestock and human consumers, as well as increasing the performance of the insects during rearing (Havenaar *et al*. 1992). Edens *et al*. (1997) reported that probiotics have the potential to enhance nutrient absorption and thereby improve growth performance and feed efficiency.

Findings of the present study revealed that fungi based protiocis *C. indica* and *S. cerevisiae* produced bigger and healthy individuals and also promoted quick larval growth of *Z.morio. S. cerevisiae* is yeast, is reported to be the best source of protein, even acting as a feeding stimulant and used as a dietary supplement, provide a number of essential vitamins and minerals to insect body. Similarly (Azaz Morales-Ramos *et al.*, 2013) reported that addition of protein to insect diet, shorten the larval development time. Prolong pupal duration was recorded in case of fungus-based diet and standard diet, the reason might be the hardening of pupal case, so pupae took longer time for emergence. No literature was traced out about prolong pupal of *Z.morio* due to tested diets. So, results of the present finding cannot be compared with work of the past. This needs further study to know the exact mechanism of loner pupal duration.

CONCLUSIONS AND RECOMMENDATIONS

The biology and nutritional profile of Zophobas morio were notably influenced by diets fermented with various probiotics. Fungal probiotics, specifically S. cerevisiae and C. indica, proved as effective as the standard Wheat bran diet, promoting maximum larval, pupal, and adult growth compared to bacterial probiotics like B. clausii and L. rhamnoses. Moreover, diets containing C. indica and S. cerevisiae significantly increased the protein content of superworms compared to both standard and bacterial probiotic-based diets. As a result, combining Wheat bran with C. indica and S. cerevisiae is recommended for mass rearing without adverse effects. Future research should focus on utilizing probiotic-based superworms as a protein supplement for improving broiler performance.

Conflict of interests

The authors declared no conflict of interest.

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